13as JORNADAS DE ANÁLISIS INSTRUMENTAL

RECINTO GRAN VIA. 14-16 NOVIEMBRE 2011

EXPOQUIMIA
Salón Internacional de la Química

Fira Barcelona

Año Internacional de la QUÍMICA 2011

SPONSORS

SEQA
Sociedad Española de Química Analítica

SECYTA
SOCIEDAD ESPAÑOLA DE CROMATOGRAFÍA Y TÉCNICAS AFINES

S.E.E.M

SOCIEDAD DE ESPECTROSCOPÍA APLICADA

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN

CTQ2011-14060-E

"In collaboration with EuCheMS-DAC"

DIVISION OF ANALYTICAL CHEMISTRY
EuCheMS

PROGRAMA CIENTÍFICO

13as JORNADAS DE ANÁLISIS INSTRUMENTAL

RECINTO GRAN VIA. 14-16 NOVIEMBRE 2011

SEQA
Sociedad Española de Química Analítica

SECYTA
SOCIEDAD ESPAÑOLA DE CROMATOGRAFÍA Y TÉCNICAS AFINES

In collaboration with EuCheMS-DAC'

DIVISION OF ANALYTICAL CHEMISTRY · EuCheMS

13as JORNADAS DE ANÁLISIS INSTRUMENTAL

LIBRO DEL CONGRESO

Barcelona
14-16 de Noviembre de 2011

APF-P09 Y. Picó ; P. Vazquez-Roig ; C. Blasco ; V. Andreu

DETERMINATION OF PHARMACEUTICALS IN WASTE WATER TREATMENT PLANT INFLUENTS. ESTIMATION OF CONSUMPTION

APF-P10 M.D. Ramos Payán ; J.A. Ocaña ; M. Villar ; M.A. Bello ; R. Fernández-Torres ; M. Callejón

COMBINACIÓN DE MICROEXTRACCIÓN EN FASE LÍQUIDA USANDO FIBRAS HUECAS (HFLPME) Y EXTRACCIÓN ENZIMÁTICA ASISTIDA MEDIANTE SONDA DE ULTRASONIDOS PARA LA DETERMINACIÓN DIRECTA DE FLUMEQUINA EN LECHE USANDO ANÁLISIS POR INYECCIÓN EN FLUJO CON DETECCIÓN LUMINISCENTE SENSITIVIZADA POR TERBIO.

APF-P11 E. Roldos ; M. Castellari ; l. Díaz ; J.A. García-Regueiro

CHANGES IN THE COMPOSITION OF EGGS TRIGLYCERIDES FROM LAY ING HENS TREATED WITH DOXYCYCLINE AND TYLOSIN.

APF-P12 M. Villar Navarro ; M. Ramos Payán ; M. Callejón Mochón ; R. Fernández Torres ; M.A. Bello López ; J.L. Pérez Bernal ; A. Guiraúm Pérez

APLICACIÓN DE LA MICROEXTRACCIÓN EN FASE LÍQUIDA UTILIZANDO FIBRAS HUECAS (HF-LPME) PARA LA DETERMINATION CROMATOGRÁFICA DE DISRUPTORES ENDOCRINOS EN AGUAS RESIDUALES URBANAS.

APF-P13 D. Zamora Zamora ; M. Blanco

CHEMOMETRICS: A VALUABLE TOOL TO IMPROVE SEPARATION EFFICIENCY IN IMS ANALYSIS

Especiación

ESP-P01 P. Bermejo-Barrera ; C. García-Sartal ; M.C. Barciela-Alonso

ARSENIC SPECIATION STUDIES IN BIOCOMPONENTS DERIVED FROM SEAWEED BY HPLC-ICP-MS

ESP-P02 N. Fabregat-Cabello ; A.F. Roig-Navarro ; J.I. García Alonso ; P. Rodríguez-González ; A. Castillo

DEVELOPMENT OF A FAST METHODOLOGY FOR THE DETERMINATION OF Cr (VI) IN SOLID MATRICES BY DOUBLE SPIKE ISOTOPE DILUTION ANALYSIS

ESP-P03 V. Funes Collado ; A. Morell-García ; J.F. López-Sánchez ; R. Rubio

STUDY OF S ELENOCOMPOUNDS IN SPROUTS GROWN IN HYDROPONIC MEDIA BY LC-ICP/MS

ESP-P04 T. García Barrera ; M. Contreras-Acuña ; J.L. Gómez-Ariza

STUDY OF ARSENIC METABOLITES IN HUMAN SERUM AND URINE AFTER SEAFOOD CONSUMPTION

ESP-P05 S. García Salgado ; D. García Casillas ; M.A. Quijano Nieto ; M.M. Bonilla Simón

ARSENIC SPECIATION IN PL ANTS BY HPLC-(UV)-HG-AFS: OPTIMISATION OF THE EXTRACTION METHOD AND APPLICATION TO NATIVE PLANTS FROM SOILS POLLUTED BY MINING ACTIVITIES

ESP-P06 C. Gilsanz ; R. Gusmao ; E. Chekmeneva ; N. Serrano ; C. Ariño ; J.M. Díaz-Cruz ; M. Esteban

ELECTROANALYSIS OF THE BINDING AND ADSORPTION OF Hg WITH SEAMINOACIDS BY DPV AND EVLS AT AU-DISK ELECTRODE

ARSENIC SPECIATION IN PLANTS BY HPLC-(UV)-HG-AFS: OPTIMISATION OF THE EXTRACTION METHOD AND APPLICATION TO NATIVE PLANTS FROM SOILS POLLUTED BY MINING ACTIVITIES

S. García Salgado, D. García Casillas, M.A. Quijano Nieto, M.M. Bonilla Simón†

Dpto. de Ingeniería Civil: Tecnología Hidráulica y Energética. E.U.I.T. de Obras Públicas. Universidad Politécnica de Madrid. C/ Alfonso XII, nº 3 y 5, 28014 Madrid, Spain.

Arsenic is a toxic metalloid widely distributed in the environment. It enters into the terrestrial and aquatic ecosystems through combination of natural processes and as a result of anthropogenic activities, such as mining activities, resulting in widespread arsenic contamination of soils and waterways. Arsenic contamination of soil and water poses a serious threat to living organisms, such as plants, which are known to accumulate arsenic in their tissues and exhibit a certain degree of tolerance. However, the estimation of arsenic toxicity requires knowledge of the individual arsenic species present in biological materials; thus, the development of convenient extraction techniques, avoiding contamination, losses and species interconversion, is needed [1, 2].

The aim of this work consisted on the development of an analytical procedure for arsenic species determination in native plant species from soils polluted by mining activities (Mónica mine, NW Madrid, Spain). The arsenic species extraction method was optimised by applying a multivariate experimental design and analysis of variance. The optimisation studies were carried out on the plant species *Thymus zygis* (root and aboveground part), using the ultrasound probe sonication and the microwave-assisted extraction, as well as different extracting agents (H_2O, H_2O-MeOH, H_3PO_4, AcOH, TMAH). From the results of the optimisation studies, arsenic species in plant samples were extracted by microwave-assisted extraction using both deionised water and 0.5 mol L^{-1} acetic acid. The method allowed us to achieve an arsenic extracted recovery between 20 and 80%, depending on the kind of plant species analysed.

Arsenic species were determined by HPLC-(UV)-HG-AFS, using both anion and cation exchange chromatography. Separations were performed on a Hamilton PRP-X100 anion exchange column, with phosphate buffer at pH 9.0 as mobile phase and using a concentration gradient from 5 to 100 mmol L^{-1}, as well as on a Hamilton PRP-X200 cation exchange column, with pyridine 2.5 mmol L^{-1} at pH 2.65 as mobile phase. The chromatographic system was coupled to the atomic fluorescence spectrometer via hydride generation, with 1.4% (w/v) $NaBH_4$ and 8.0 M HCl, with a previous photo-oxidation step, which employs a UV lamp and a solution of potassium persulfate. The developed method by anion exchange chromatography led us to the separation of four arsenosugars (glycerol, phosphate, sulfate and sulfonate sugars), AsB, As(III), DMA, MMA and As(V) in less than 17 minutes, with LODs between 5 and 22 pg of As (100 µL sample injection volume). On the other hand, the species As(III), As(V), AsB, TETRA y TMAO were separated in less than 14 minutes, by the cation exchange method, with LODs between 26 and 53 pg of As. The methods were applied to ten different plant species, which contained mainly As(V) (2-675 µg g^{-1}), and As(III) at lower concentration levels (0.1-9 µg g^{-1}). Furthermore, MMA (0.5-0.15 µg g^{-1}) and TMAO (0.14-0.16 µg g^{-1}) were only detected in the plant species *Corrigiola telephiifolia* and *Holcus mollis*, respectively.

[1] M.J. Ruiz-Chancho, J.F. López-Sánchez, E. Schmeisser, W. Goessler, K.A. Francesconi, R. Rubio. *Chemosphere*, 2008, 71, 1522–1530.
[2] L. Jedynak, J. Kowalska, J. Harasimowicz, J. Golimowski. *Science of the Total Environment*, 2009, 407, 945-952.

Tipo de comunicación: Poster

ARSENIC SPECIATION IN PLANTS BY HPLC-(UV)-HG-AFS: OPTIMISATION OF THE EXTRACTION METHOD AND APPLICATION TO NATIVE PLANT SPECIES FROM SOILS POLLUTED BY MINING ACTIVITIES

S. García Salgado, D. García Casillas, M.A. Quijano Nieto and M.M. Bonilla Simón

Dpto. de Ingeniería Civil: Tecnología Hidráulica y Energética. E.U.I.T. de Obras Públicas. Universidad Politécnica de Madrid. C/ Alfonso XII, 3. 28014 Madrid, Spain. E-mail: sara.garcia@upm.es. Tel: +34 913367755; Fax: +34 913367958

INTRODUCTION

Arsenic is a toxic metalloid widely distributed in the environment. It enters into the terrestrial and aquatic ecosystems through combination of natural processes and as a result of anthropogenic activities, such as mining activities, resulting in widespread arsenic contamination of soils and waterways. Arsenic contamination of soil and water poses a serious threat to living organisms, such as plants, which are known to accumulate arsenic in their tissues and exhibit a certain degree of tolerance. However, the estimation of arsenic toxicity requires knowledge of the individual arsenic species present in biological materials; thus, the development of convenient extraction techniques, avoiding contamination, losses and species interconversion, is needed [1, 2].

[1] M.J. Ruiz-Chancho, J.F. López-Sánchez, E. Schmeisser, W. Goessler, K.A. Francesconi, R. Rubio. Chemosphere (2008) 71, 1522–1530.
[2] L. Jedynak, J. Kowalska, J. Harasimowicz, J. Golimowski. Sci. Total Environ. (2009) 407, 943–953.
[3] García-Salgado, S.; García-Casillas, D.; Quijano-Nieto, M.A.; Bonilla-Simón, M.M. Water Air Soil Pollut. 2011 (DOI 10.1007/s11270-011-0882-x).

PROCEDURE

PLANT SAMPLE

- MINERALIZATION (0.25 g)
  1. MW digestion (200 °C for 10 min) with 10 mL of HNO_3/H_2O_2 (1:1; v/v)
  2. Dilution to 50 mL with H_2O
  - As_T determination by ICP-AES [3]
- EXTRACTION (0.20 g)
  1. MAE at 90 °C for 5 min, with 8 mL of H_2O and AcOH 0.5 M (3 extraction steps)
  2. Centrifugation at 15000 *g* for 10 min
  3. Mixture of the 3 extraction steps, dilution to 25 mL and centrifugation
  - As_T extracted determination by ICP-AES
  - As species determination by HPLC-(UV)-HG-AFS

OBJECTIVES

The aim of this work consisted on the development of an analytical procedure for arsenic species determination in native plant species from soils polluted by mining activities (Mónica mine, NW Bustarviejo, Madrid, Spain). The arsenic species extraction method was optimised by applying a multivariate experimental design and analysis of variance. Arsenic species were determined by HPLC-(UV)-HG-AFS, using both anion and cation exchange chromatography.

PLANT SAMPLES

The plant samples studied in this work were:

- *Arrhenatherum album*
- *Corrigiola telephiifolia*
- *Cynosorus echinatus*
- *Digitalis thapsi*
- *Holcus mollis*
- *Jasione montana*
- *Plantago lanceolata*
- *Rumex acetosella*
- *Thymus zygis*
- *Trisetum ovatum*

Root (R) and Aboveground part (AP)

EXPERIMENTAL

OPERATING CONDITIONS FOR As SPECIATION BY HPLC-(UV)-HG-AFS

HPLC	
Pump	PU-2089 plus (Jasco)
Anion exchange: Column	Hamilton PRP-X100 (Phenomenex)
Mobile phase (concentration gradient)	Phosphate buffer 5, 20 and 100 mmol L^{-1} pH 9.0
Cation exchange: Column	Hamilton PRP-X200 (Phenomenex)
Mobile phase (isocratic)	Pyridine 2.5 mmol L^{-1}, pH 2.65 (HCl)
Flow rate	1.0 mL min^{-1}
Injection volume	100 µL
On line photo-oxidation	
UV lamp	10.570 UV Cracker (PS Analytical)
$K_2S_2O_8$: Anion exchange	2% (w/v) in 2% (w/v) NaOH (0.5 mL min^{-1})
Cation exchange	4% (w/v) in 4% (w/v) NaOH (0.5 mL min^{-1})
Hydride generation	
HCl	8 mol L^{-1} (1.4 mL min^{-1})
$NaBH_4$	1.4% (w/v) in 0.1 mol L^{-1} NaOH (1.4 mL min^{-1})
Ar (GL separator) flow rate	250 mL min^{-1}
Air (drying) flow rate	2.5 L min^{-1}
AFS instrument	
Detector	Millennium Excalibur (PS Analytical)
Wavelength (nm)	193.7
Primary current (mA)	27.5
Boost current (mA)	35.0
Gain	1, 10 and 100

TABLE 1. Factors and levels considered in the full factorial design.

Extraction method	Factor	Level		
		Low	Central	High
MAE [a]	Temperature (°C)	50	70	90
	Time (min)	2.5	5	7.5
	Extraction steps	1	2	3
UPS [b]	Amplitude (%)	10	20	30
	Time (min)	2.5	5	7.5
	Extraction steps	1	2	3

[a] Microwave-assisted extraction
[b] Ultrasound probe sonication

ACKNOWLEDGEMENTS: This work was financially supported by Universidad Politécnica de Madrid (project 188/Q105815-102) and Ministerio de Educación y Ciencia (project CTM2007-66432). The authors are grateful to Dr. Pastor Piñeiro and Dr. Gonzaga García-Montero, for helping in the collection and identification of plant samples and to Dr. Luis Jordá Bordehore and Mr. Rafael Jordá Bordehore for information on the mine site, as well as to the Excelentísimo Ayuntamiento de Bustarviejo for admittance to the mine zone and the facilities offered.

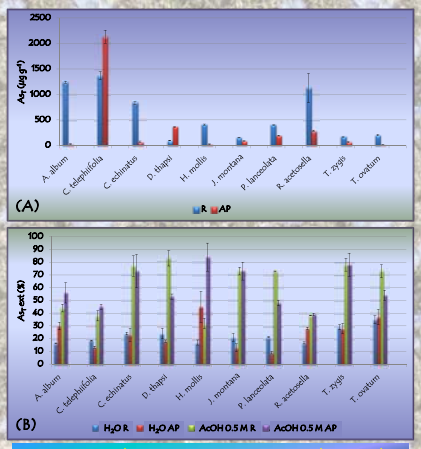

FIGURE 1. Total As concentration (µg g^{-1}) in plant samples after MW digestion (n=3) (A) and total As extracted (%) after MAE (90 °C, 5 min, 3 extraction steps, n=3) (B), obtained by ICP-AES determination.

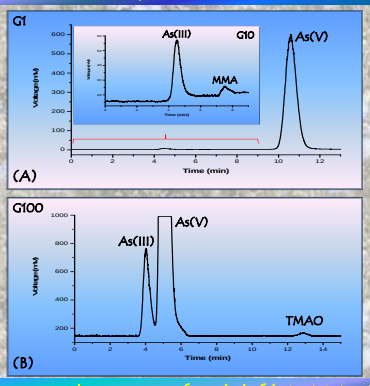

FIGURE 2. Chromatograms of *C. telephiifolia* (AP) (A) and *H. mollis* (R) (B) AcOH 0.5M extracts from anion and cation exchange, respectively, by HPLC-(UV)-HG-AFS.

RESULTS

TABLE 2. Optimised extraction conditions and maximum total As extracted (%) for MAE and UPS.

Thymus zygis (0.2 g), 8 mL of H_2O				
	MAE		UPS	
	As_T ext (%)	Optimised extraction conditions	As_T ext (%)	Optimised extraction conditions
R	34	90 °C 7.5 min 3 extraction steps	24	30% 7.5 min 3 extraction steps
AP	36	90 °C 2.5 min 3 extraction steps	15	30% 7.5 min 3 extraction steps

TABLE 3. Total As extracted (%) obtained by optimised extraction conditions for MAE, using different extracting agents (n=3).

Thymus zygis (0.2 g), MAE, 90 °C, 5 min, 3 extraction steps, 8 mL of extracting agent										
	As_T ext (%)									
	H_2O	H_2O/MeOH			H_3PO_4		AcOH		TMAH	
		(9:1)	(1:1)	(1:9)	0,1 M	0,5 M	0,1 M	0,5 M	0,1 M	0,5 M
R	29 ± 2	23 ± 4	16 ± 3	16 ± 3	77 ± 4	80 ± 5	56 ± 5	78 ± 5	80 ± 3	92 ± 3
AP	28 ± 4	26 ± 3	21 ± 2	18 ± 3	73 ± 2	79 ± 4	57 ± 3	78 ± 9	54 ± 4	83 ± 8

TABLE 4. Results obtained for As species (µg g^{-1} As) by HPLC-(UV)-HG-AFS after MAE with AcOH 0.5 M (n=3).

PLANT		As (µg g^{-1})				Σ As species (µg g^{-1})	Column recovery (%)[a]
		As(III)	As(V)	MMA	TMAO		
A. album	R	4.6 ± 0.3	352 ± 19	n.d.	n.d.	357 ± 19	65 ± 5
	AP	0.48 ± 0.03	10.5 ± 0.8	n.d.	n.d.	11.0 ± 0.8	78 ± 13
C. telephiifolia	R	7.5 ± 0.6	188 ± 13	0.64 ± 0.01	n.d.	196 ± 13	38 ± 6
	AP	5.5 ± 0.4	612 ± 24	0.74 ± 0.01	n.d.	618 ± 24	65 ± 4
C. echinatus	R	1.9 ± 0.2	34 ± 3	n.d.	0.13 ± 0.01	36 ± 3	5.6 ± 0.8
	AP	0.14 ± 0.02	27 ± 2	n.d.	n.d.	27 ± 2	52 ± 9
D. thapsi	R	0.4 ± 0.1	31 ± 3	n.d.	n.d.	31 ± 3	39 ± 5
	AP	2.4 ± 0.2	142 ± 8	n.d.	n.d.	144 ± 8	75 ± 5
H. mollis	R	2.2 ± 0.1	74.1 ± 0.9	n.d.	0.16 ± 0.01	76.5 ± 0.9	59 ± 6
	AP	0.50 ± 0.02	10.0 ± 0.6	n.d.	n.d.	10.5 ± 0.6	52 ± 8
J. montana	R	1.74 ± 0.03	54 ± 2	n.d.	n.d.	56 ± 2	51 ± 3
	AP	0.55 ± 0.04	33.3 ± 0.7	n.d.	n.d.	33.9 ± 0.8	53 ± 5
P. lanceolata	R	9.5 ± 0.6	141 ± 5	n.d.	n.d.	150 ± 5	52 ± 2
	AP	0.71 ± 0.03	52.2 ± 0.6	n.d.	n.d.	52.9 ± 0.6	56 ± 3
R. acetosella	R	5.7 ± 0.4	174 ± 0.7	n.d.	n.d.	179.7 ± 0.8	41.7 ± 0.3
	AP	2.8 ± 0.1	48 ± 2	n.d.	n.d.	51 ± 2	45 ± 2
T. zygis	R	0.63 ± 0.05	66 ± 5	n.d.	n.d.	67 ± 5	52 ± 5
	AP	0.91 ± 0.08	33 ± 2	n.d.	n.d.	34 ± 2	65 ± 8
T. ovatum	R	0.81 ± 0.04	72 ± 4	n.d.	n.d.	73 ± 4	49 ± 4
	AP	0.14 ± 0.02	4.1 ± 0.3	n.d.	n.d.	4.2 ± 0.3	76 ± 9

n.d. = not detected
[a] Calculated as the ratio of the sum of the As species with the total As extracted

CONCLUSIONS

- The developed method by anion exchange chromatography led us to the separation of four arsenosugars (glycerol, phosphate, sulfonate and sulfate sugars), AsB, As(III), DMA, MMA and As(V) in less than 17 minutes, with LODs between 5 and 22 pg of As, whereas the species As(III), As(V), AsB, TETRA y TMAO were separated in less than 14 minutes, by the cation exchange method, with LODs between 26 and 53 pg of As.
- The chemometric approach used for As extraction method optimisation allows the characterisation of interdependence of different variables as well as the reduction of the number of experiments necessary for successful method optimisation. The factors and levels considered for the optimisation of the microwave-assisted extraction method (MAE) and the ultrasound probe sonication based extraction method (UPS) are summarised in Table 1.
- Optimisation experiments were carried out on *Thymus zygis*, using 0.2 g of sample and 8 mL of deionised water. The optimised extraction conditions for MAE and UPS are shown in Table 2. The best results were obtained for MAE with 90 °C, 2.5 or 7.5 min, and 3 extraction steps. Finally, we decided to apply 5 min of heating in order to use the appropriate extraction conditions both for roots and aboveground parts of plants samples.
- The optimised MAE method were applied on *Thymus zygis* using different extracting agents (H_2O, H_2O/MeOH, H_3PO_4, AcOH, TMAH) (Table 3). From the results, arsenic species in plant samples were extracted by MAE method using both deionised water and 0.5 mol L^{-1} acetic acid. The method allowed us to achieve an arsenic extracted recovery between 20 and 80%, depending on the kind of plant species analysed (Figure 1B).
- As(V) was the major species found in the plant samples analysed, followed by As(III) at much lower concentration levels. Furthermore, MMA was only detected in the plant species *C. telephiifolia* (AP) (Figure 2A), and TMAO in *C. echinatus* (R) and *H. mollis* (R) (Figure 2B).